IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	)	Examiner: M. WOODWARD
ROGER P. EKINS	)	
Serial No.07/984,264	)	Group Art Unit: 1813
Filed: 1 December 1992	)	
For: DETERMINATION OF AMBIENT CONCENTRATION OF SEVERAL ANALYTES.	)	

DECLARATION OF PROFESSOR IVAN MAURICE ROITT

Commissioner of Patents
and Trade Marks
Washington, DC 20231

Sir:

I, Ivan Maurice Roitt, declare as follows:

1. I am Professor and Head of the Department of Immunology at the University College and Middlesex School of Medicine, London. I worked in research in the immunology and immunodiagnostics field since 1953. I am a Fellow Of The Royal Society of the United Kingdom. My curriculum vitae and list of academic publications accompanies this declaration.

2. I am familiar with the above patent application and the work of the inventor, Professor Roger Ekins in the field of immunoassay and, in particular, his "ambient analyte" methodology. I am familiar with the objections raised by the Examiner in the Office Action of 23 August 1993 and have read the art cited by the Examiner, namely Ekins WO84/1031 ('031) and Chang US 4591570 ('570).

3. I have been asked to comment on the argument presented by the Examiner in the above noted Office Action that the above application would have been obvious to a person of ordinary skill in the art in view of Ekins '031 and Chang '570.

4. As noted on page 2 of the above application, competitive immunoassays are typically carried out by those skilled in the art in accordance with the work of Berson and Yalow. Berson and Yalow recommended that maximum sensitivity in assays is achieved when 30-50% of the analyte in a sample is bound. Non-competitive assays are usually carried out using an excess of binding agent to bind close to 100% of the analyte in a sample.

These approaches are widely accepted in the field and accordingly, immunoassays are typically carried out using large amounts of binding agent.

5. Chang '570 represents an example of a prior art non-competitive assay. The assay disclosed in Chang attempts to bind all of the analyte (red blood cells) in a sample applied to a matrix of spots containing immobilised antibody. The aim in Chang is to fill the spots to maximum capacity, using multiple antibodies to bind individual red blood cells.

In the Office Action, the Examiner argues that Chang operates under the "ambient analyte" conditions described in Ekins '031. However, the Examiner's argument overlooks the fact that multiple antibodies bind to each red blood cell in Chang and since each antibody is fixed to a solid phase, each red cell is bound to the solid phase by multiple links. In view of this, the

overall affinity constant the antibodies for the red blood cells is given by multiplying the individual affinity constants (K) of each antibody link. If a red cell is attached to the solid phase by n antibody links, the overall affinity constant will be K^n. Thus, in Chang '570, the effective affinity constant K is very much larger than that suggested by the Examiner, with the result that the amount of binding agent used in Chang is much greater than the 0.1V/K moles requirement of the above application.

I therefore believe that Chang '570 certainly does not teach or suggest Professor Ekins' ambient analyte methodology to the person of ordinary skill in the art.

6. As regards Ekins '031, this reference does not teach the skilled person the advantages of using less than 0.1V/K moles of binding agent. In example 2 of Ekins '031 the use of antibody having affinity constant of 2×10^{10} $lmol^{-1}$ (in an amount having a binding capacity of 10 fmoles) with samples of volume 0.2, 0.4 and 0.8mls, represents the use of V/K, 0.5V/K and 0.25V/K moles of binding agent. These amounts are all in considerable excess of the more stringent 0.1V/K moles requirement of the above noted application.

In addition, Ekins '031 requires the user of the assay to have an idea of the expected concentration of the analyte in a liquid sample so that he or she can ensure that an amount of binding agent is used which only binds an insignificant proportion of the analyte in the sample. The above application has the considerable practical advantage over '031 as the use of less than 0.1V/K moles of binding agent means that a small amount of analyte is removed from the total, <u>irrespective of the analyte</u>

concentration.

I believe that because people skilled in the art would intuitively be convinced that extremely small amounts of binding agent would be swamped by analyte, they would not normally even bother to apply the Laws of Mass Action to calculate the fractional occupancy as the above application has done; frankly, I was astonished that these microspots provided a workable system with the advantages set out above and am quite convinced that the disclosure of Ekins '031 would not have led the person of ordinary skill in the art to reduce further the amount of binding agent used in the assay of Ekins '031, especially in light of the prevailing view in the field, and certainly not to the realisation of the advantages set out above.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001, Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of this application or any patent issued thereon.

Declared by .....
Professor Ivan Maurice Roitt

Date ..... 24 Nov 1993 .....

DECLARED by the above-named at Imperial House, 15-19 Kingsway, London WC2, England, this 24th day of November 1993 before me:

Notary Public of London, England
My Commission expires with life